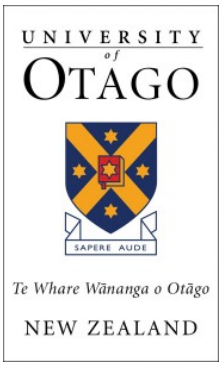
Delay activity in the Wulst of pigeons *(Columba livia)* displays neural correlates for both memory and reward.

Ethan Marrs



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Abstract

The avian brain has two main visual pathways: the tectofugal pathway, that terminates in the Entopallium, and the thalamofugal pathway, that terminates in the avian Wulst. The Wulst is a parasagittal bulge located on the avian telencephalon, and is considered to be homologous to mammalian Striate cortex. Previous electrophysiology studies of the avian visual system have only recorded from the Entopallium, in which neurons displayed evidence of sample coding. We recorded single-unit activity from the Wulst of four birds trained on a delayed matching-to-sample (DMS) procedure. Two birds were trained and tested with differential outcomes (DO), in which only one sample stimulus was rewarded for a correct response; and two birds were trained and tested with common outcomes (CO), where both sample stimuli were rewarded. We expected delay activity from the Wulst would differ from baseline activity for both stimuli, and across both types of DMS task. For the CO DMS task, we found that excitatory and inhibitory delay activity differed from baseline activity following both sample stimuli. However, for the DO DMS task, we found inhibitory delay activity deviated from baseline activity following both rewarded and unrewarded sample stimuli. Whereas, excitatory delay activity only differed from baseline following the rewarded sample stimulus, and not the unrewarded stimulus. From this, it appears that the avian Wulst may be involved in both memory and reward coding.

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Thank you, next.

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List of Abbreviations

DMS	**Delayed Matching-to-Sample**
ITI	**Inter-trial Interval**
IT	**Inferior Temporal**
MTS	**Matching-to-Sample**
DR	**Delayed Response**
HD	**Hyperstriatum Dorsale**
HIS	**Hyperstriatum Intercalatus Superior**
IHA	**Intercalatus Hyperstriati Accessorii**
HA	**Hyperstriatum Accessorium**
CO	**Common Outcomes**
DO	**Differential Outcomes**
CED	**Cambridge Electronic Design**
ENTO	**Entopallium**
NCL	**Nidopallium Caudolaterale**
Hz	**Cycles Per Second**
NA	**Not Applicable**
μm	**Micrometre**
mm	**Millimetre**
AP	**Anterior-Posterior**

ML	**Medial-Lateral**
DV	**Dorsal-Ventral**
h	**Height**
l	**Length**
w	**Width**

Chapter 1

Introduction

1.1 Delayed Matching-to-Sample

Birds have a remarkable ability to successfully complete complex tasks involving learning and memory. Like primates, a bird's ability to perform these complex tasks have often been measured using the delayed matching-to-sample task (DMS) (Anderson & Colombo, 2019, Johnston, Anderson & Colombo, 2016). The DMS procedure proceeds typically as follows: the subject is presented with a fixation period leading into an initial inter-trial interval (ITI) followed by a sample stimulus, for instance, an image of a dog or a horse. Once a response to the sample stimulus occurs the image is removed, and a delay period occurs. After the delay period, the subject is presented with both the dog and a horse images as comparison stimuli. The correct response is to respond to the comparison stimulus that matched the sample. Correct responses are rewarded, whereas incorrect responses are not rewarded and sometimes result in a punishment, for example a high-pitched tone. Both correct and incorrect responses are followed by the ITI and the start of the next trial. An example of a typical trial on a DMS task is shown in Figure 1.1.

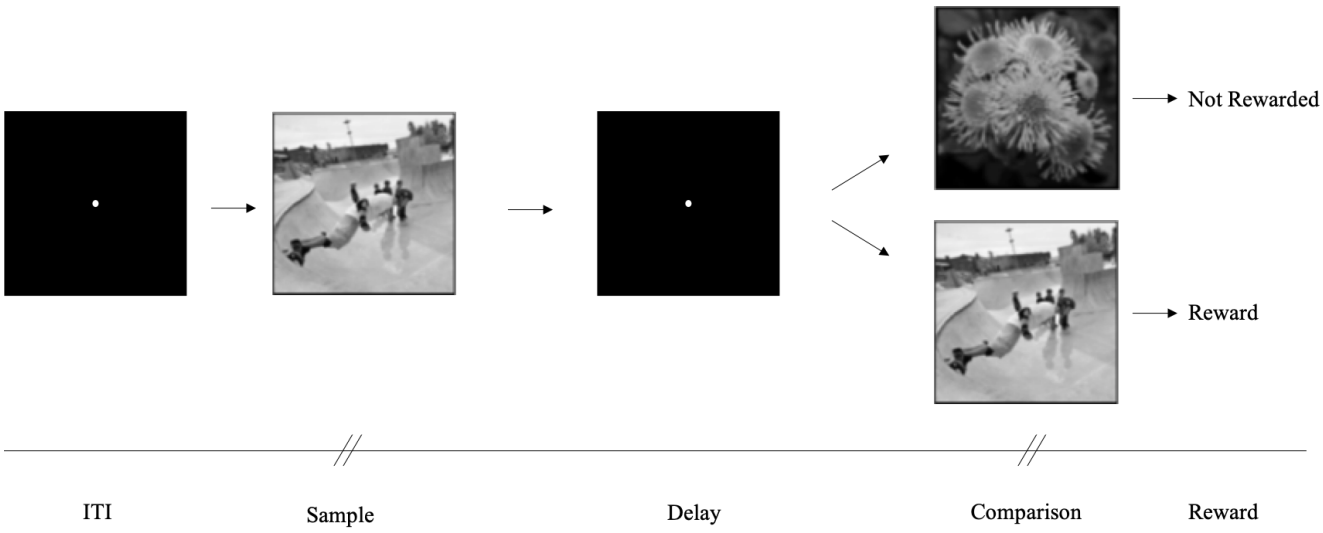

Figure 1.1: DMS task used within the present study, showing the Inter-trial interval (ITI), Sample, Delay, Comparison and Reward phases. Note for this example flower would not be rewarded but skateboarder would, as skateboarder was the sample stimulus.

To accurately perform the DMS task the subject must remember the sample stimulus presented across the delay period. Fuster and Jervey (1981), were the first to show that neurons within the Inferior Temporal cortex (IT), a higher-order visual system region within primates (Gross, 1972, Logothetis, Pauls, Poggio, (1995), are able to fulfil this role.

1.2 Inferior Temporal Cortex and Memory

Fuster and Jervey (1981) trained nine macaque monkeys to perform a DMS task. The monkeys sat in a primate chair where they faced a projected response panel. Trials were initiated with the presentation of the sample stimulus, the sample stimulus was either a colour or a combination of colour and symbol, positioned centre of the panel, at eye level. The sample stimulus remained on the screen until a touch response was made onto the sample. Once touched, the sample disappeared, and the delay initiated. After a delay varying between 6 to 32 seconds (usually 18 seconds during recording), two or four comparison

stimuli appeared. Touching the matching comparison stimulus of the original sample was the correct response and therefore rewarded. The position of the sample stimuli was counterbalanced during the comparison stimuli presentation, thus ensuring the monkey had to remember the sample across the delay.

Of the neurons recorded from within the IT cortex, the majority displayed an increase in firing rate to the sample, with some being specific to either colour, or symbol, or a colour-symbol combination, an unsurprising finding given that the IT cortex is primarily a visual area. Neurons that elicited a response during the sample period were often reactivated during the comparison period. Within the lower bank portion of the Temporal Sulcus, a large number of neurons produced trains of spikes either partially or completely across the delay, irrespective of sample stimulus, as shown in Figure 1.2. The single neuron recordings exhibited within the study are consistent with other studies, which show that IT neurons are important for memory.

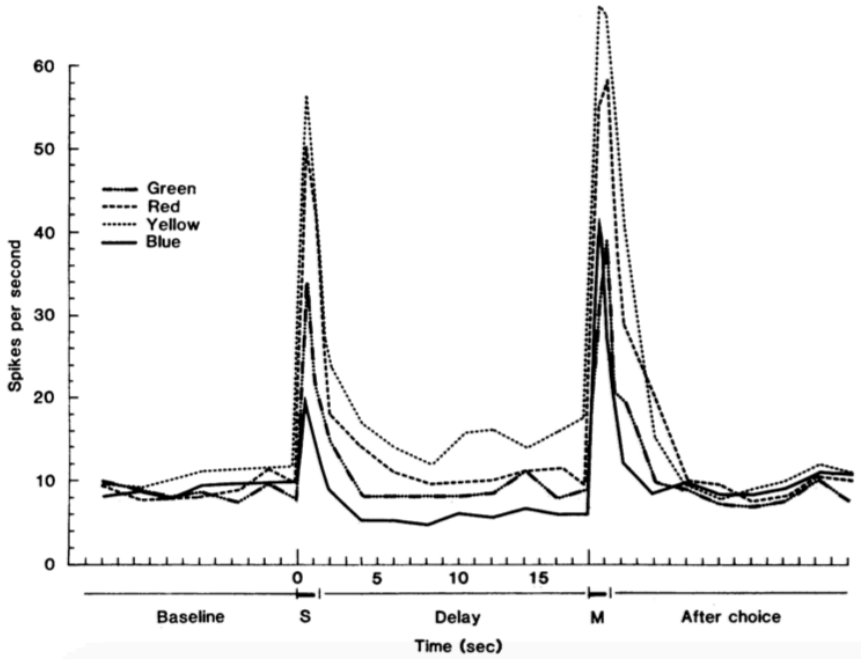

Figure 1.2 Average spike frequencies of a neuron within the IT cortex during DMS for each of the four tested colours. S represents the time when the sample stimulus was delivered, whereas, M represents the comparison period is delivered. (Adapted from Fuster and Jervey, 1981).

Fuster, Bauer and Jervey (1981), showed the effects of IT cortex cooling on visual memory tasks. Four male macaque monkeys were first trained to perform the simultaneous matching-to-sample (MTS) task, then DMS, and finally the delayed response (DR) task. What separates the DMS task and a DR task is that in a DMS task, the correct response stimulus can appear in different locations to the sample, however in a DR task the correct response stimulus always reappears in the same location as the sample. For all tasks, monkeys were sat in a primate chair where they faced a projected response panel. Firstly, the simultaneous MTS task was used. The MTS was initiated through the presentation of a sample stimulus. When the sample stimulus is responded to, two comparison stimuli would appear underneath the original sample. The two comparison stimuli were projected onto separate buttons, pressing the matching comparison stimuli to the sample stimulus was correct and therefore rewarded. Failure to respond or selecting the non-matching comparison stimulus resulted in no reward and extinction of the trial. After the simultaneous MTS training, the monkeys were trained on the DMS. The DMS task is almost identical to the previous simultaneous MTS, however when a response is made to the sample stimulus, it is removed, and a delay occurs. After the delay, the comparison stimuli would appear, and the monkey would have to respond to the stimulus originally presented as the sample to be rewarded. Failure to respond or selecting the non-matching comparison stimulus again resulted in no reward and extinction of the trial.

The final task the monkeys were trained on was the DR task. For this task, each trial starts when a one of two buttons becomes illuminated by a white light. Once the illuminated button has been touched the light disappears, and the delay begins. After the delay, both buttons become illuminated and the monkey has to select the originally lit button to gain the reward. The illuminated button randomly changed across trials and the delay increased as training increased.

After training was completed across all tasks, the monkeys underwent surgery. Gold plated copper cooling tubes were implanted bilaterally on the surface of the IT cortex. A heat extraction device attached to the tubes was used during testing, which reduced the IT cortex temperature to twenty degrees Celsius. This temperature did not result in any obvious abnormalities in behaviour, sensorium, dexterity or motivation.

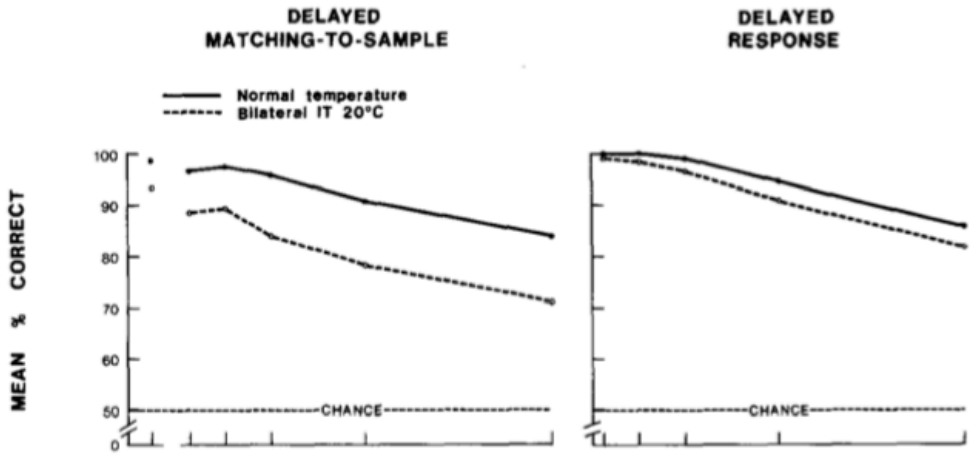

Figure 1.3: Comparison between normal temperature and bilateral cooling of the IT, across both the DMS and the DR task. Horizontal axis shows the effect temperature has across a delay(from Fuster, Bauer, Jervey, 1981)

Only a small impairment was seen when the monkeys performed the simultaneous MTS task, indicating that both the monkeys' visual perception and discrimination were undisturbed through cooling. Whereas, during the DMS task cooling caused a significant impairment in performance, as shown in the left of figure 1.3. Fuster, Bauer, and Jervey stated that the cooling tubes had caused an effect on the memory processes across the delay. Unlike the DMS findings, the DR task performance was not impacted through cooling. The authors concluded that this may have been a result of the monkeys' using non-visual memory strategies to solve the task. Both unilateral and bilateral cooling of the IT cortex produced deficits in memory, however it was greater in bilateral cooling. The findings indicate that the IT cortex cooling negatively affected the visual memory in monkeys.

IT cortex neurons also hold information about complex objects. Miyashita and Chang (1988) trained two monkeys to perform a delayed non-matching-to-sample task. Each trial consisted of a sample stimulus which was paired with two comparison stimuli, one that matched the sample and one that did not. The sample stimuli were selected from a pool of 100 different computer-generated patterns and 100 scenery images (examples shown in Figure 1.4). After the presentation of one sample stimulus, one of the comparison stimuli would be presented after a delay. If the comparison stimulus matched the sample, the monkey had to continue pressing the lever for the trial to finish. However, if the comparison was different from the sample stimulus, the monkey had to release the lever and touch the screen, thus obtaining reward. Both sample and comparison stimuli were presented for 0.2 seconds with a 16 second delay in between. Extracellular recordings of neuronal activity were taken during the task from the Anterior Ventral Temporal cortex.

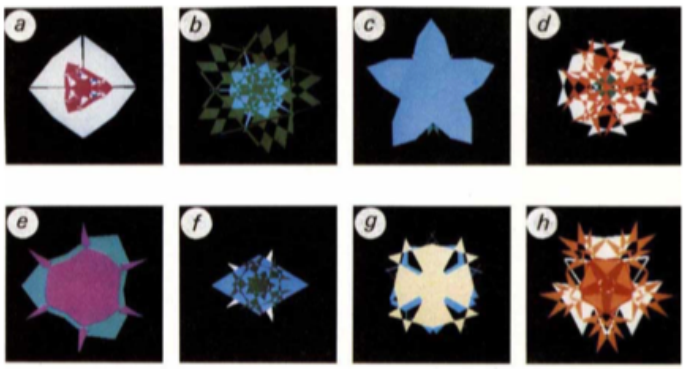

Figure 1.4 Examples of the computer-generated stimuli used within the delay non-matching to sample task. (from Miyashita and Chang, 1988)

A total of 188 neurons were recorded across the task, of which 144 exhibited a change of activity during at least one period of the trial. Of the 144 neurons, 95 displayed either a decrease or increase of activity during the delay period. They found that the neuronal activity did not decline across the entire

delay period (shown in Figure 1.5), and delay activity was not always linked with sample stimulus presentation. That is, delay activity did not continue from the initial onset of the sample, but rather delay activity started a few seconds after the sample was removed. Miyashita and Chang (1988) therefore argue that the delay activity was not residual sensory activity from the presentation of the stimulus, but instead represented holding visual information in working memory.

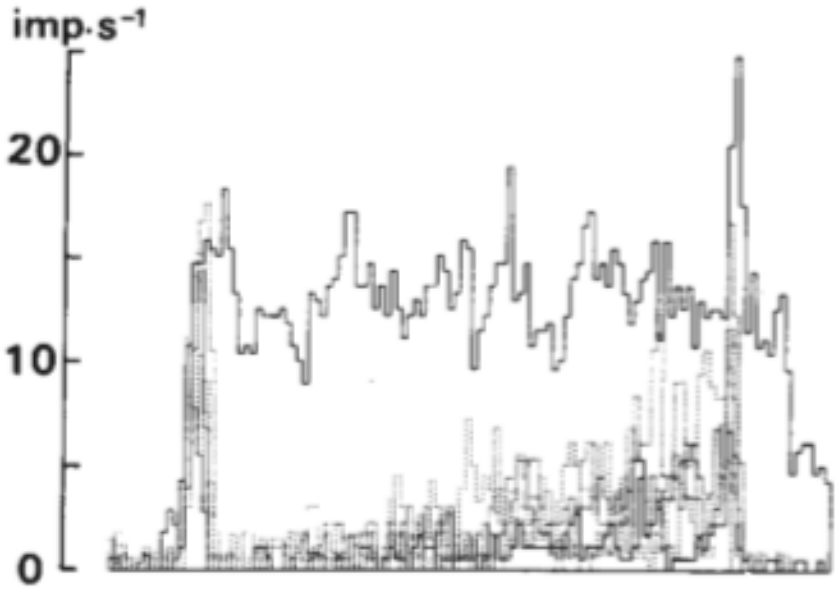

Figure 1.5 Neuronal spike activity of a neuron across the delay period, presented through a density histogram showing impulses per second (imp.s-¹) . (from Miyashita and Chang, 1988)

Previous to Fuster and Jervey (1981), the IT cortex was thought to be exclusively for higher order visual tasks, however as shown above, the IT cortex neurons exhibit delay activity which represents the ability to hold information for later use. Delay activity is not limited to primates however, as it has been shown in many animal groups including birds.

1.3 The Avian Brain

Karten and Hodos (1967) produced the first stereotaxic atlas of the pigeon brain, which was the first attempt at mapping and giving nomenclature to the different areas of the avian brain. Naturally due to advancements in science, this atlas has become outdated and new nomenclature has been given to different areas within the avian brain (Reiner et al. 2004). Originally, the Karten and Hodos nomenclature described the avian telencephalon as hypertrophied striatum, essentially homologous to the mammalian basal ganglia. The updated nomenclature, on the other hand, more accurately describes the telencephalon as a neocortex-like structure comprised of pallial tissue, homologous to that of mammals. (Güntürkün, 2005; Reiner et al. 2004)

It is widely recognised that primates and birds both have an incredibly sophisticated and complex visual system. Despite primates and birds diverging from one another around 300 million years ago, their visual pathways are extremely similar. All amniotes possess two main visual pathways, which span from their retina to their telencephalon (Shimizu and Bowers, 1999).

1.3.1 Tectofugal and Thalamofugal Pathways

The primate tectofugal pathway starts at the retina, passing through the superior colliculus then the thalamus, finishing within the extrastriate cortices (shown in Figure 1.6). Similarly, the avian tectofugal pathway starts at the retina, passing through the optic tectum, then the nucleus rotundus, finishing at the Entopallium within the dorsal ventricular ridge (shown in Figure 1.6). The tectofugal pathway of primates has been shown to be critical for many visual motor actions, for example the maintenance of orientation and attention to stimulus presentation (Shimizu & Bowers, 1999; Bischof & Watanabe, 1997).

The primate thalamofugal pathway begins at the retina, passing through the lateral geniculate nucleus and finishing within the striate cortex (also shown in Figure 1.6). The avian thalamofugal pathway shares a similar pathway,

where it starts at the retina, passing through the optic nuclei of the thalamus, finishing at the Wulst within the telencephalon (also shown in Figure 1.6). The thalamofugal pathway of primates is important for visual discriminations, such as detection of fine details and colour (Shimizu & Bowers, 1999).

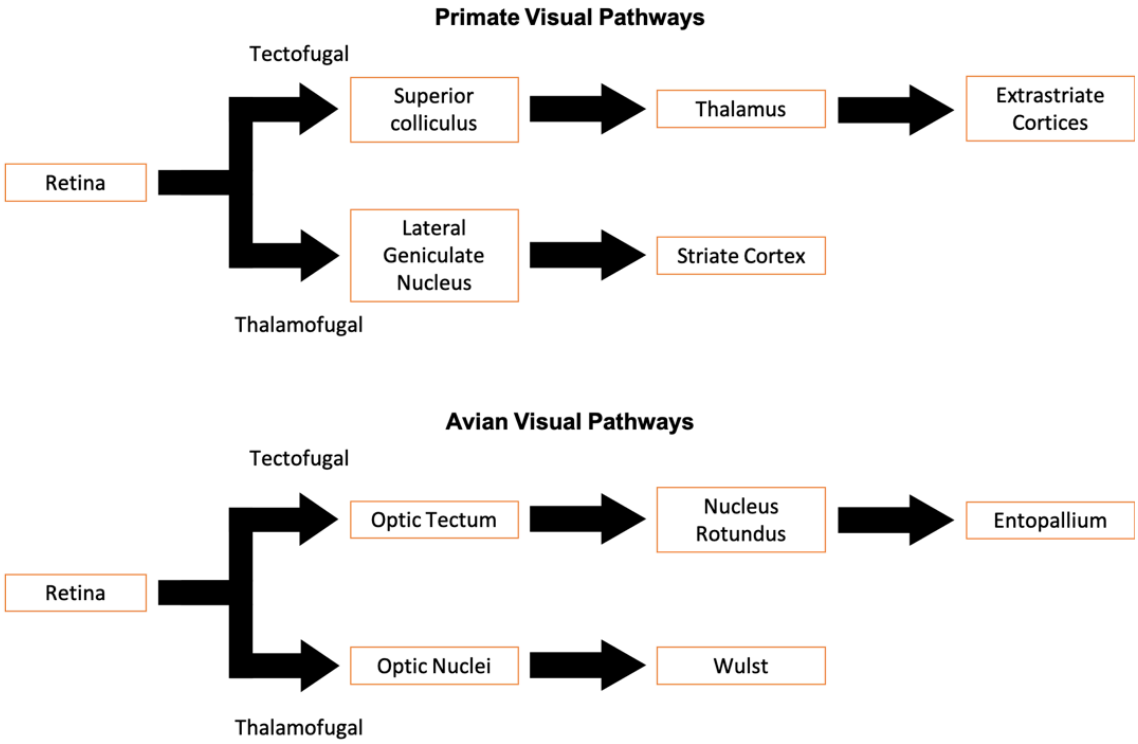

Figure 1.6 Differences between the two primate and two avian visual pathways.

Despite a plethora of anatomical, physiological and chemical similarities, the thalamofugal pathways do differ between the primate and avian species. For example, the primate thalamofugal pathway is more developed and easily identifiable from their tectofugal pathway, whereas the avian thalamofugal pathway is not as developed nor as identifiable from their tectofugal pathway, especially in lateral eyed birds such as pigeons (Shimizu & Bowers, 1999). Therefore, the visual dominance of the avian tectofugal pathway suggests it is functionally equivalent to the primate thalamofugal pathway. Surprisingly the avian tectofugal and the primate thalamofugal pathways fail to share a common amniote source, rather the similarities are likely a result of convergent

evolution. These similarities point toward a need for such parallel processing to effectively process complex visual information. (Shimizu & Bowers, 1999).

1.4 The Wulst

The avian Wulst is a small but complex parasagittal bulge in the dorsal aspect of the pallium of birds (Karten & Hodos, 1967; see new avian brain nomenclature, Reiner et al., 2004; see Figure 1.7). The Wulst is dividable into two main areas, the posterior visual and the anterior Somatosensory area (Karten, Hodos, Nauta & Revzin, 1973). Being the telencephalic target of the thalamofugal visual pathway, the visual Wulst is also regarded as the equivalent to the mammalian IT cortex (Karten et al, 1973). For example, the neurons in both the Wulst and IT cortex have small receptive fields which have displayed retinotopic mapping (Cowey, 1964; Miceli, Gioanni, Reperant, & Peyrichoux, 1979; Revzin, 1969). The visual aspect of the Wulst is made up of four layers: Firstly, the Hyperstriatum Dorsale (HD); secondly, Hyperstriatum Intercalatus Superior (HIS); thirdly, Hyperstriati Accessorii (IHA); and finally, the Hyperstriatum Accessorium (HA) (Atoji & Wild, 2019; Karten et al, 1973; Wild & Williams, 2000; Wylie, Glover, Lau, 1998).

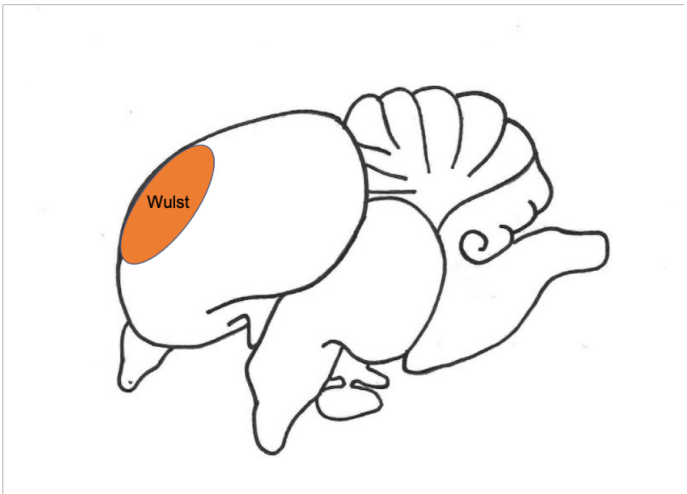

Figure 1.7 Orange section indicates the Wulst region of the avian brain (Adapted from Koenen, Pusch, Bröker, Thiele, & Güntürkün, 2015).

Pasternak (1977) investigated the role of the Wulst in memory through the use of the DMS task. Eight pigeons, of which five were experimentally naïve were trained on a DMS task. Naïve pigeons were first auto-shaped to peck the response screen, once this was achieved training on the DMS occurred. Pigeons were initially trained on a 0 second delay trial, where once the sample had been responded to the sample would disappear and two comparison stimuli (one matching to sample and one different) would appear. Once the naïve pigeons were successfully achieving a 90% accuracy rate across three sessions, the delay would increase. Progressively increasing delays of 1, 2, 4 and 8 seconds were introduced to the DMS task, granted the pigeons were continuing to reach the 90% threshold across successive sessions.

Once achieving 90% across the 8 second delay, the pigeons were introduced to a simultaneous matching trial. In the simultaneous matching trials, once the sample had been responded to once, two comparison stimuli (one matching to sample and one different) were to appear either side of the sample stimulus. The other three experimentally sophisticated pigeons were only trained on the simultaneous matching trials. After a pigeon had completed the simultaneous DMS task with 90% accuracy across three sessions, the pigeon was introduced to the mixed DMS task. In the mixed DMS task the pigeon was re-introduced to all previous levels of delay of the DMS (0, 1, 2, 4, 8) and simultaneous condition. Random presentation of each delay occurred but were counter-balanced across the session. After a week of the mixed DMS task, performance levels of each pigeon were taken from multiple sessions and averaged, to obtain a control.

Following the control recording, the pigeons underwent a sham surgery. During the sham surgery, the pigeons were anesthetized, their skull was opened, and some dura matter was removed, however no lesion was made. Following recovery from the sham surgery, the pigeons were again tested on the mixed DMS for multiple sessions, and their performance recorded. Following the mixed DMS testing sessions, the pigeons underwent the true

surgery where the Wulst was lesioned. After the surgery, the pigeons were re-trained until criterion was met across DMS levels. Following criterion, all pigeons were retested on the mixed DMS task.

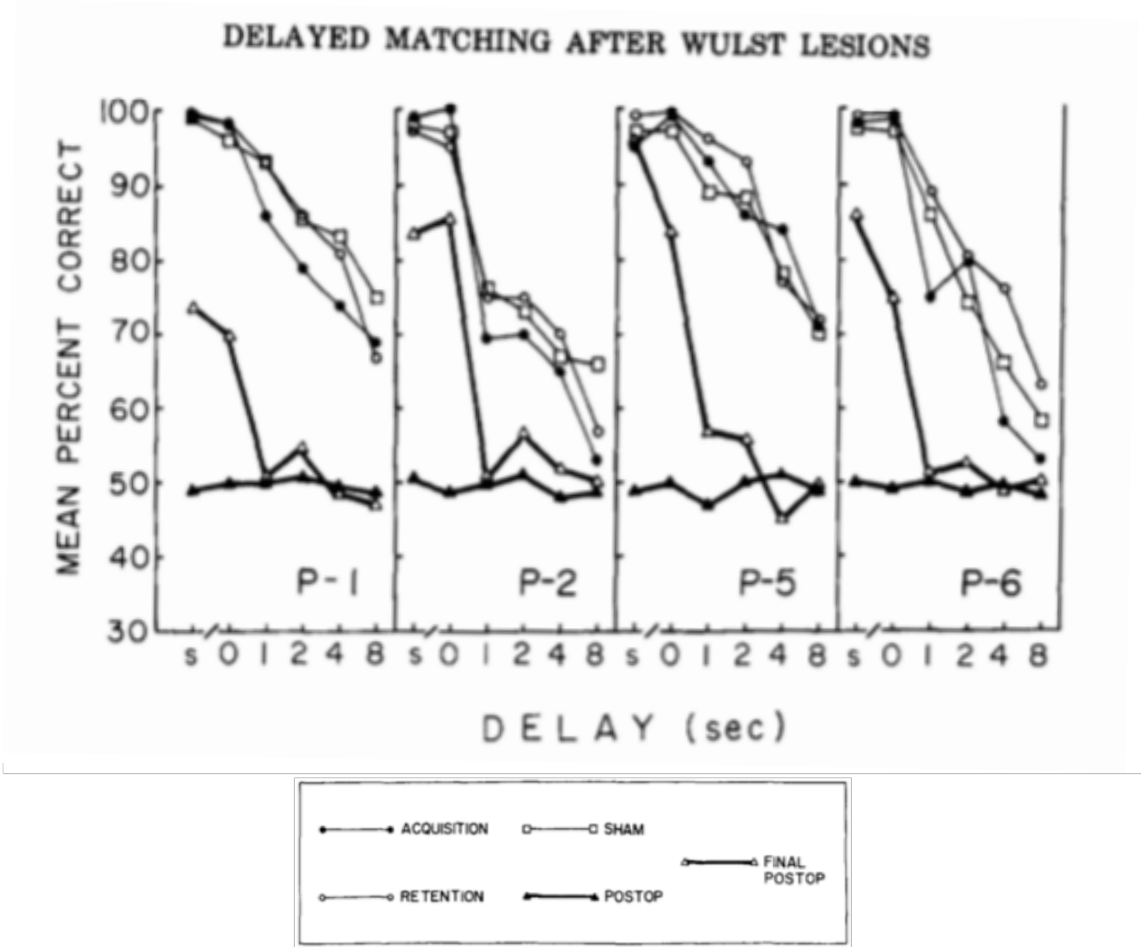

Figure 1.8 Accuracy performance data across several pigeons (P1, P2, P5, P6), at each stage of testing for the DMS task. (Adapted from Pasternak, 1977)

On the post-operative test following the true surgery, all pigeons exhibited a significant accuracy decrease across all levels of delay and simultaneous matching aspects of the mixed DMS (see Figure 1.9). However, it should be noted that the pigeons developed a preference towards the yellow stimulus, indicating the lesion did not interfere with the pigeons' ability to discriminate the stimuli. Only two of the pigeons were able to re-achieve criterion on the 0 second delay condition during retraining, however when tested in a mixed DMS task this accuracy again fell below criterion (see Figure

1.9). The significant accuracy drops across all pigeons between the simultaneous matching and the delay levels, including 0 second delay, indicates the crucial role the Wulst possesses in working memory.

Likewise, to the IT cortex, the Wulst was originally thought to be exclusively for higher order visual tasks (Karten & Hodos, 1967), however as shown above Wulst neurons also exhibit delay activity. In the current study, we look to explore the delay in an effort to gain further understanding into what the activity is.

1.5 The Current Study

The primary aim of the current study is to further our understanding of the delay properties of neurons in the avian Wulst. Specifically, we will compare and contrast the neuronal properties of neurons of the Wulst during a CO DMS task and DO DMS task. All previously mentioned studies have used CO DMS tasks. During a CO DMS task, selecting the comparison stimulus that matched the original sample for that trial produces the same reward irrespective of the stimulus (i.e. horizontal line or vertical line; Fuster & Jervey, 1981; Fuster, Bauer & Jervey, 1981; Miyashita & Chang, 1988). Conversely, in DO, the reward varies for each of the sample stimuli used. For example, successful pairing of a trial which the stimulus is a square produces a food reward, whereas successful pairing of a trial where the stimulus is a circle produces no reward. An unsuccessful pairing still results in a punishment (Johnston, Anderson, Colombo, 2016; Trapold, 1970). While the CO DMS task is generally used to test visual working memory mechanisms (as the pigeon must remember the sample in each trial to obtain the rewarded), the DO DMS will highlight whether neurons in Wulst are modulated by reward (as only the skateboard trials will be rewarded). It is vital that research is now undertaken surrounding the Wulst, as previously the available technology was limited, such as in Pasternak, (1977).

Four pigeons will complete either a CO or DO DMS task, whilst undergoing electrophysiological recordings from the Wulst. Our hypothesis is that delay activity within the Wulst will differ from baseline for both stimuli across both types of DMS task. We expect that for the CO DMS task, delay activity will deviate from baseline across the entire length of the delay following both sample stimuli, indicating that the Wulst is involved in visual working memory. For the DO DMS task, delay activity will only deviate following the rewarded sample stimulus across the entire length of the delay, but not the unrewarded stimulus, indicating that the Wulst is sensitive to reward information.

Chapter 2

Method

2.1 Subjects

Four experimentally sophisticated pigeons (*Columba livia*) served as the subjects for the current experiment. Pigeons were housed separately in a colony room, that was maintained at 20ºC and had a light/dark cycle of 12 hours with lights on at 7am. Pigeons were weighed daily before testing, their body weights were maintained on a mixture of grain, corn, and peas. The amount of food given to pigeons was adjusted daily, this was to ensure pigeons were maintained at 80-85% of their free feed body weight. Thus, ensuring efficient performance during the experiment. Grit and water were available to the pigeons continually. The handling and the care of the pigeons was carried out in accordance with the University of Otago Code of Ethical Conduct for the Manipulation of Animals (Ethics number: 51/16001).

2.2 Apparatus and Stimuli

An operant chamber internally measuring 35l x 43w x 39h cm was used during both training and electrophysiological testing. At the front of the chamber was a 17-inch monitor, which was used to display the stimuli. A Perspex panel with six square holes measuring 60 mm by 60 mm was positioned in front of the monitor. The holes were arranged in a two (rows) x three (column) grid and were 65 mm apart from centre to centre. The stimuli were only presented in the top row of holes. The sample stimulus was displayed in the middle hole and the comparison stimuli were displayed in the side holes, see Figure 2.1. Black and white photographs of a flower and a skateboarder served as the two

stimuli, see Figure 2.2. Colour photographs were not used in this study as black and white photographs have been shown to provide a same result (Herrnstein & Loveland, 1964).

Figure 2.1: Display seen by the pigeon during trials, showing skateboarder as the sample stimulus.

An infrared touch frame that records the XY coordinates of all pecks was positioned between the Perspex panel and monitor. A grain hopper was positioned underneath the floor directly in front of the centre of the screen and 110 mm below the lower centre hole. The hopper rose to floor level following a correct trial. A light located by the hopper was illuminated during the presentation of grain. Grain was used as the reward.

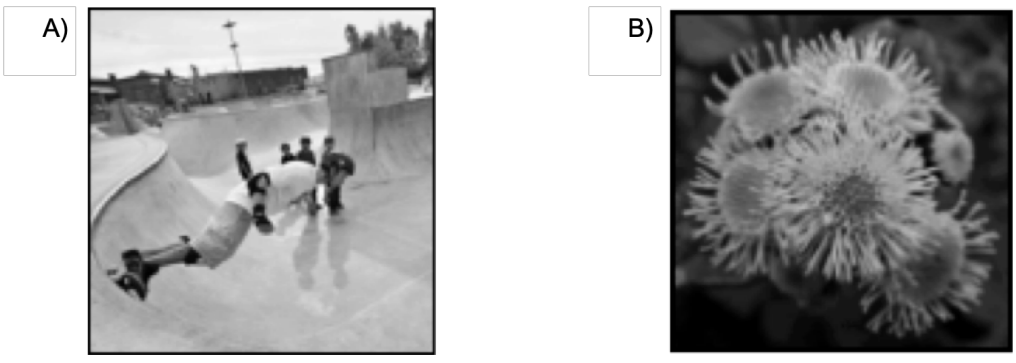

Figure 2.2: Stimuli used within the experiment; A) Skateboard, and B) Flower.

2.3 Training Protocol

In their previous experiment the pigeons were magazine trained to eat food from the hopper before being autoshaped to peck a stimulus (a white dot). Autoshaping is the process where you teach a animal to respond to a stimulus, normally when a response is made the animal is rewarded. Once the pigeons were reliably responding to the dot, they were trained through classical conditioning to respond to the skateboarder and flower stimuli used in the current experiment. Once they were reliably pecking the skateboarder and flower stimuli, they were trained on the DMS task. When the pigeon was responding with at least 80% accuracy with a 0-second delay between sample and comparison stimuli, the delay was increased, first to a .5-second delay, then a 1-second delay, and finally a 3-second delay. The criterion for advancing from one delay to the next was at least 80% accuracy on the task, as per the labratories achievement criterion. Training process took an average of two months, of which the pigeons were trained five times a week.

2.4 Behavioural Task

The process for both DO and CO procedure for both stimuli is shown below in Figure 2.3. Each trial began with a 10-second inter-trial interval (ITI). Following the ITI the sample stimulus (skateboarder or flower) was displayed in the centre hole. Three pecks to the sample stimulus removed the stimulus and initiated the delay period. A 3-second delay period was selected as it has been shown the produce a measurable delay effect in pigeons (Johnston, Anderson, Colombo, 2016). After the 3-second delay period, the pigeon was presented with the two comparison stimuli (skateboarder and flower) displayed in the outer two holes. A correct response required that the pigeon peck the

comparison stimulus that had appeared as the sample. When the skateboarder stimulus served as the sample, a correct pairing resulted in the grain hopper illuminating and a reward consisting of 2.5-seconds of access to grain.

The task differs at this point depending on which reward condition the pigeons were placed in (i.e. DO or CO). For the pigeons within the DO reward scheme when the flower stimulus served as the sample, correct responses resulted in the hopper illuminating but no reward, whereas for pigeons within the CO reward scheme when the flower stimulus served as the sample, correct responses resulted in the standard illumination of the hopper and 2.5-second access to grain. Incorrect responses to either the comparison stimulus for both DO and CO pigeons were punished with 1-second 500Hz tone at 65 decibels, followed by the ITI. A correction routine was in place in training such that all incorrect trials were repeated until a correct response was made. Punishments were included so that the pigeons were aware of an incorrect pairing, and thus were able to change their behaviour on the following correction trial.

Each session consisted of 64 trials. Within each session, the skateboarder stimulus served as the sample for half of the trials and for the other half the flower stimulus served as the sample, this presentation was counterbalanced. The left and right position of the comparison stimuli was balanced across trials with skateboarder on the left and flower on the right for half the trials, and the opposite arrangement for the other half of the trials, also was counterbalanced.

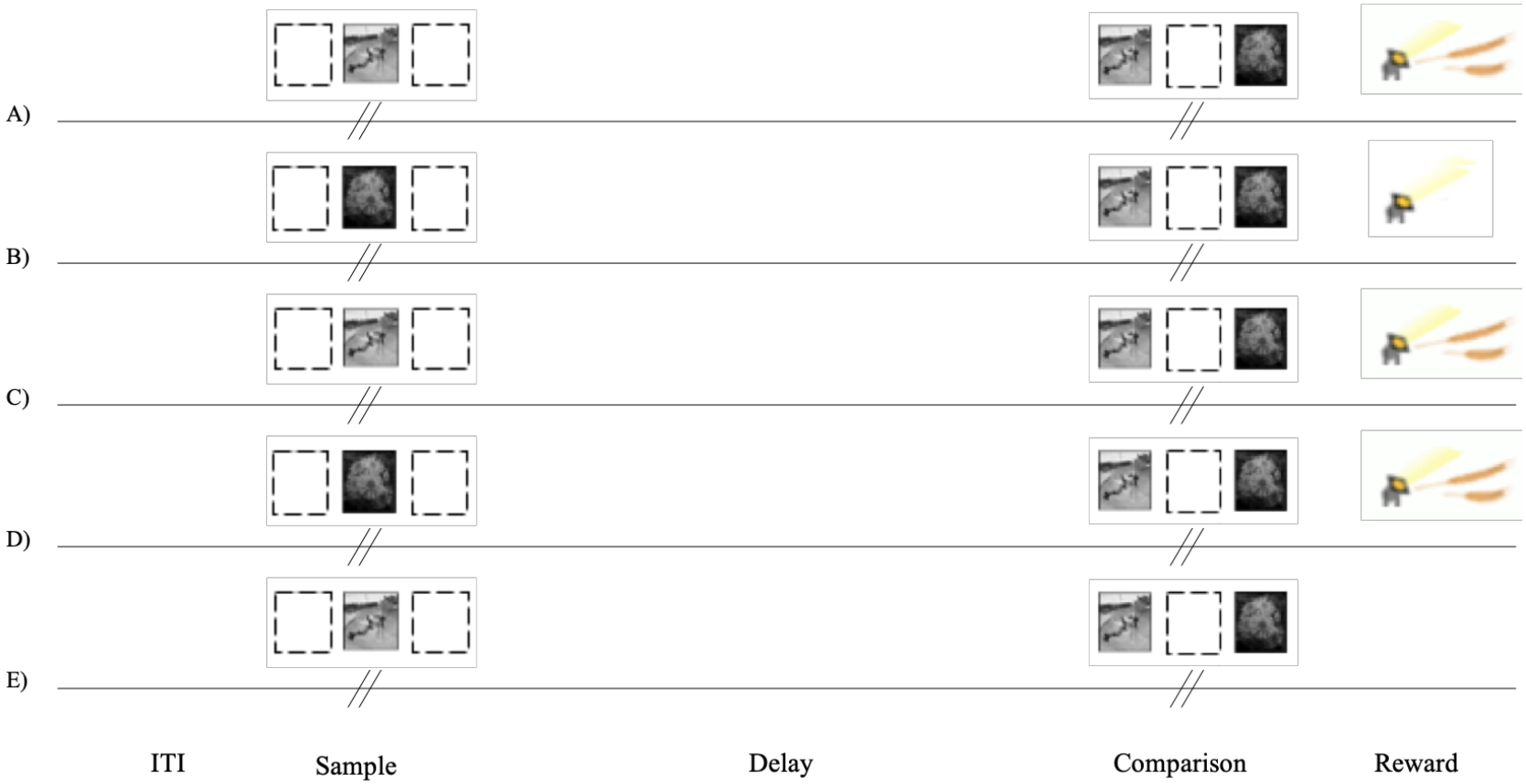

Figure 2.3: Procedure for the DMS task, showing both skateboarder and flower trials for DO and CO. A) DO where skateboard served as sample stimulus, B) DO where flower served as sample stimulus, C) CO where skateboard served as sample stimulus, D) CO where flower served as sample stimulus, and finally E) CO or DO trial where the behavioural task is not correctly performed (note the lack of reward). Seen above the reward section of the DMS task is two icons, the left being a light, to represent the light that was shined onto the hopper, and the other a grain, to represent the food reward in each successful trial excluding the DO flower trials.

2.5 Surgery

Once the pigeons were reliably completing the task with at least 80% accuracy with a 3-second delay, surgery was performed to install microdrives used for the neuronal recording. All four pigeons had microdrives installed into the Wulst. Two pigeons received a microdrive in the left hemisphere, while the other two received it the right hemisphere. Pigeons M6 and M8 had their microdrive positioned at AP +11.0, ML +3.0 and DV 0.5; pigeon M10's microdrive was positioned at AP +11.0, ML -3.0 and DV 0.7; and finally pigeon M11 had its microdrive positioned at AP +11.0, ML -3.0 and DV 1.0 (Note: AP,ML, and DV are sterotaxic atlas coordinates, where AP is Anterior-Posterior, ML is Medial-Lateral, and finally Dorsal-Ventral)(Karten & Hodos, 1967).

For surgery, the pigeons were initially injected with a mixture of Ketamine (54 mg/kg) and Xylazine (6 mg/kg). Feathers on the head were then removed. The pigeons were placed in a Revzin Stereotaxic Adapter (Karten & Hodos, 1967) to immobilise the head and a topical anaesthetic (10% Xylocaine) was applied to the scalp. The skin covering the skull was retracted exposing the skull and six stainless steel screws were inserted into the skull. One of these served as the ground screw. A hole was drilled above the Wulst area and the dura was removed. A microdrive (Bilkey, Russell, & Colombo, 2003) housing the electrodes was lowered into the hole until the tips of the electrodes were positioned just above the Wulst area. The microdrive was secured to the skull using dental acrylic and the wound was closed through the use of sutures. Xylocaine was reapplied before the pigeons were placed into a padded and heated recovery cage. The pigeon stayed in the recovery cage and monitored closely until it had returned to an active state and then returned to their home cage where they were given another seven days to recover before retraining and recording began. All surgical tools, implanting equipment, surgical area

and recovery cages were sterilized in an effort of safe practice prior to the start of each surgery.

2.6 Neuronal Recording

The microdrive contained eight 25 μm Formvar-coated nichrome wires (California Fine Wire, Grover Beach, CA, USA) used to measure single neuron activity. On each testing session, we combed for activity on any one of the eight wires and used one of the remaining wires as the indifferent. The indifferent wire is used as a reference wire, the wire is selected based on which wire emitted the least noise. The signals were amplified (10,000%) and filtered using a GrassP511K (Grass Instruments, Quincy, MA, USA) (Low pass 300Hz, High pass 1KHz). The only selection criterion was that the isolated neuron had a signal-to-noise ratio of no less than 2:1. Once the neuron had been isolated, the behavioral task began. A CED (Cambridge Electronic Design, Cambridge, UK) electrophysiology system with Spike2 software was used to store and analyze the data. A separate computer that was used to control the behavioral task also sent codes to the CED system to align key task events.

Following each recording session where we recoded from single neurons, the electrodes were moved approximately 40 μm deeper into the Wulst before the pigeon was returned to their home cage. If we did not record from any neural activity, the electrodes were moved approximately 20 μm deeper into the Wulst and the animal returned to its cage. Recording sessions took approximately one hour to complete (this included locating a viable neuron, and completion of the 64-trial task). Pigeons completed one session daily for five days each week, for several months (3 – 6 months dependent on the individual bird).

When the electrodes reached the end of the Wulst, the final recording position was marked by sending a 9V current through each electrode for 10 seconds, thereby creating an electrolytic lesion at the tip of each electrode. The pigeons

were then euthanized and perfused with physiological saline and 10% formalin.

2.8 Data Analysis

All data recorded was firstly transformed in DosBox 0.74. DosBox organized the raw spike data into visual and numerical data, of which could then be entered into IBM SPSS statistics version 24.0 for data analysis. Afterwards, the data was transferred into Microsoft Excel and converted into graphs. For behavioral analysis, we conducted a two-tailed paired t-test with stimulus (skateboard vs flower) to find the difference in correct responding for each individual pigeon. Whereas, for neuronal analysis, delay neurons were determined by comparing the neuronal activity from the 3-second delay period, with the average activity from the middle 5-seconds of the ITI period for the skateboard trials using Bonferroni corrected paired t-tests ($p < .05$). A significant difference in activity between the ITI and delay period indicated that it was a delay neuron. A neuron was categorized as "excitatory" if the activity in the delay activity increased compared to the ITI, and "inhibitory" if activity decreased during the delay period relative to ITI activity. Neurons that failed to last for the entire recording session, or that had an average fire rate of less than 0.3Hz during a session were filtered out of final analysis. Data were normalized across the individual sessions by dividing the maximum activity value of that sessions ITI.

Chapter 3

Results

3.1 Behavioural Data

The behavioural performance of the pigeons averaged across all tested sessions is shown in Table 1. All four pigeons achieved a high level of accuracy, across the DMS task using the skateboard and flower stimulus.

Table 3.1. Individual pigeon average accuracy performance across the DMS task.

Pigeon	*Skateboard accuracy*	*Flower accuracy*
M6 (CO)	90.7%	87.7%
M11 (CO)	85.5%	87.7%
M8 (DO)	92.9%	76.3%
M10 (DO)	100%	89.8%

We compared each pigeons' skateboard and flower performance across all tested sessions using paired student t-test (Bonferroni corrected)(df=sessions). With respect to the DO pigeons, both M8, $t(21)=7.16$, $p<.001$, and M10, $t(35)=7.430$, $p<.001$, displayed better performance on rewarded skateboard trials compared to the non-rewarded flower trials, this was most likely due to the skateboard trials being associated with the possibility of reward. With respect to the CO pigeons, on the other hand, there was little difference between

performance on skateboard and flower trials for either M11, $t(39)=1.70$, $p=.098$, or M6, $t(17)=2.09$, $p=.052$, although the difference fell short of significance for M6. M6's small neuron count was not due to quality of available neurons, but rather cells were sparse and not often able to be located, despite multiple attempts by multiple trained people.

3.2 Total Number of Neurons

We recorded from a total of 125 neurons. Of the 125 neurons, 63 were obtained from pigeons within the DO procedure, and 62 were from CO procedure pigeons. Filtering neurons reduced the DO pigeons neuron amount to 52, and the CO pigeons neuron amount to 49. Of the DO procedures 52 neurons, 24 had a significant change of activity during the skateboard delay period. Of the CO procedures 49 neurons, 32 had a significant activity change during either the skateboard or flower delay period. Neurons were only analysed for the DO procedure if they showed significant change in the skateboard trials, as this was the only rewarded stimulus. Whereas for the CO procedure neurons were analysed if they showed significant change in either skateboard or flower trials.

Neurons were classified as either excitatory or inhibitory delay neurons. A neuron was characterized as 'excitatory' if average firing rate increased in comparison to the ITI. Whereas, neurons were characterized as 'inhibitory' if average firing rates across the delay decreased relative to the ITI. The different types of delay neurons are shown within Table 3.2. Of the 24 DO delay neurons, 16/24 responded exclusively to skateboard, either in an excitatory (en, 8/24) or inhibitory manner (in, 8/24). 2/24 DO neurons responded to the different stimuli in different ways, with some being excitatory to skate but inhibitory to flower (ei, 1/24), and the others being inhibitory to skate but excitatory to flower (ie, 1/24). Finally, 6/24 neurons responded to both stimuli in an inhibitory manner (ii, 6/27).

Table 3.2. Rate and corresponding percentage of the different types of delay activity in the Wulst across both DO and CO procedures. The first letter of the pair (e.g. en) corresponds to the delay firing rate for when the skateboard served as the sample stimulus, and the second letter (e.g. en))corresponds to the delay firing rate when the flower served as the sample stimulus. For reference, n is a non-delay neuron, e is an excitatory neuron and i is an inhibitory neuron. For example, an en neuron is a neuron that responds to the skateboard stimulus in an excitatory manner, whilst not responding to the flower stimulus in any manner.

Skateboard and Flower

	en	in	ei	ie	ee	ii	ne	ni	**Total**
DO	8	8	1	1	0	6	NA	NA	**24**
DO %	33.3%	33.3%	4%	4%	0%	25%	NA	NA	
CO	1	4	0	0	13	8	5	1	**32**
CO %	3%	12.5%	0%	0%	40.6%	25%	15.6%	3%	

For the 32 CO delay neurons, 5/32 responded exclusively to skateboard, either in an excitatory (en, 1/32) or inhibitory manner (in, 4,32). Unlike DO, in no instance did a CO neurons respond in an excitatory manner to one stimuli, but in an inhibitory manner to the other (ei/ie, 0/32). 21/32 neurons responded to both stimuli in the same way, either excitatory to both stimuli (ee, 13/32) or both inhibitory (ii, 9/32), Finally 6/32 neurons responded exclusively to flower, either in an excitatory (ne, 5/32) or inhibitory manner (ni, 1/32).

Both excitatory and inhibitory delay activity for DO and CO procedures are shown below in Figure 3.1. On the top left (Figure 3.1A) is a DO neuron that fired during the delay in an excitatory manner, after the skateboard stimulus, and then failed to fire during the delay after the flower stimulus. On the top

right (Figure 3.1B) is another DO neuron that fired during the delay in an inhibitory manner, after the skateboard stimulus, and after the flower stimulus. On the bottom left (Figure 3.1C) is a CO neuron that fired during the delay in an excitatory manner, after the skateboard stimulus, and then also fired during the delay in an excitatory manner, after the flower stimulus. On the bottom right (Figure 3.1D) is another CO neuron that fired during the delay in an inhibitory manner, after the skateboard stimulus, and also fired during the delay in an inhibitory manner, after the flower stimulus.

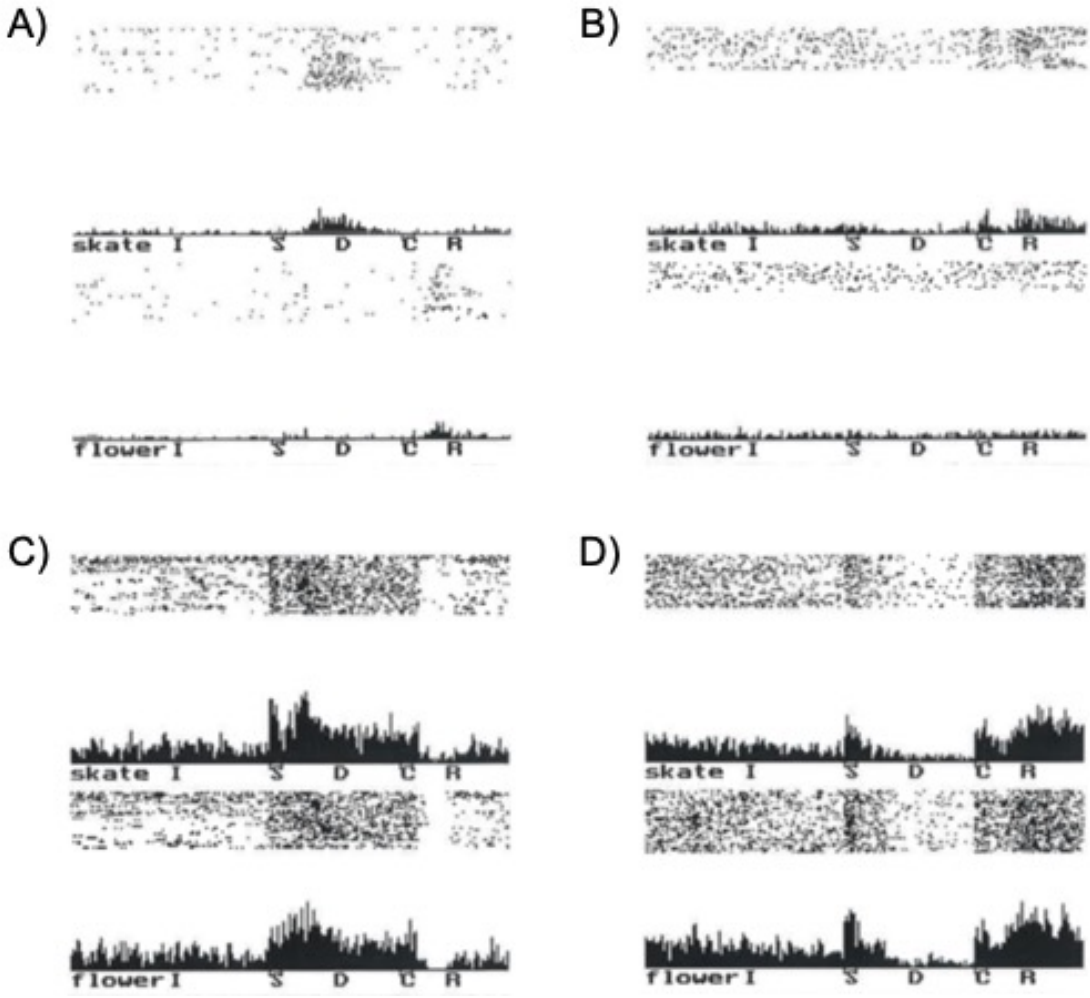

Figure 3.1. Examples of Wulst neuron excitatory or inhibitory activity for DO and CO procedure. Horizontal axis displays the time period in which the neuron response occurs, I–ITI, S–Sample, D-Delay, C-Comparison, R-Reward. (A) DO neuron (en) with excitatory activity during skateboard and non-delay activity during flower trial; (B) DO neuron (ii) with inhibitory activity during both skateboard and flower trials; (C) CO neuron (ee) with excitatory activity during skateboard and during flower trial; (D) CO neuron (ii) with inhibitory activity during skateboard during flower trial.

3.3 Population Response Profiles

To create population profiles, we compared the neuronal activity of delay neurons from skateboard trials to the neuronal activity of those same neurons when the flower served as the sample stimulus for both the DO and CO procedures. For each response profile five separate two-way repeated-measures ANOVAs (Greenhouse-Geisser corrected) were conducted with bin (99, 6, 60, 6, 50 50-ms bins for the ITI, sample, delay, comparison and reward periods respectively) and stimulus (skateboard vs flower) as factors. A stimulus effect during a certain period means a significant effect between the two stimuli (skateboard and flower) occurred, whereas a bin effect during a certain period means a significant change occurred across the bins themselves (decrease or increase), and finally a stimulus x bin interaction effect means a significant change occurred between the stimuli across bins.

3.3.1 Excitatory DO Activity

The response profile for the excitatory delay neurons (ee, ei, en) of the DO condition is shown in the Figure 3.2. In the ITI period, there was no significant effects of stimulus, $F(1,8)=0.71$, $p=0.43$, bin $F(98,724)=0.95$, $p=0.47$, and no stimulus x bin interaction, $F(98,784)=0.99$, $p=0.45$. In the sample period, there was a significant effect of stimulus, $F(1,18)=7.43$, $p<0.03$. There was no significant effects of bin, $F(5,40)=1.56$, $p=0.23$, or stimulus x bin interaction $F(5,40)=1.72$, $p=0.18$. The significant effect of stimulus in the sample period was expected and is likely due to the fact that the skateboard stimulus predicts that a reward is possible, whereas the flower stimulus predicts the absence of a reward.

In the delay period, stimulus produced a significant effect, $F(1,8)=44.72$, $p>.001$. There was no significant effect of bin, $F(59,472)=2.21$, $p=0.07$, and no stimulus x bin interaction $F(59,472)=1.27$, $p=0.29$. The significant effect of stimulus in the delay indicates that there is a difference between the delay activity of skateboard and flower trials. This was explored further through a comparison with the baseline ITI activity, for which a significant difference was observed for skateboard, $t(8)=4.09$, $p<.001$ but not for flower, $t(8)=.50$, $p=.63$, this difference was expected due to the potential reward associated with skateboard.

In the comparison period, there was a significant effect of stimulus, $F(1,8)=5.99$, $p<.040$. No significant effects of bin $F(5,40)=0.46$, $p=0.67$, or stimulus x bin interaction $F(5,40)=0.48$, $p=0.70$, occurred. Finally, in the reward period, stimulus produced a significant effect, $F(1,8)=5.42$, $p<.048$. There was no significant effects of bin $F(49,392)=1.39$, $p=0.26$, or stimulus x bin interaction $F(49,392)=1.19$, $p=0.36$. The significant effect of stimulus in both the comparison and reward was expected and is likely due to the pigeons' neural activity being governed by upcoming reward.

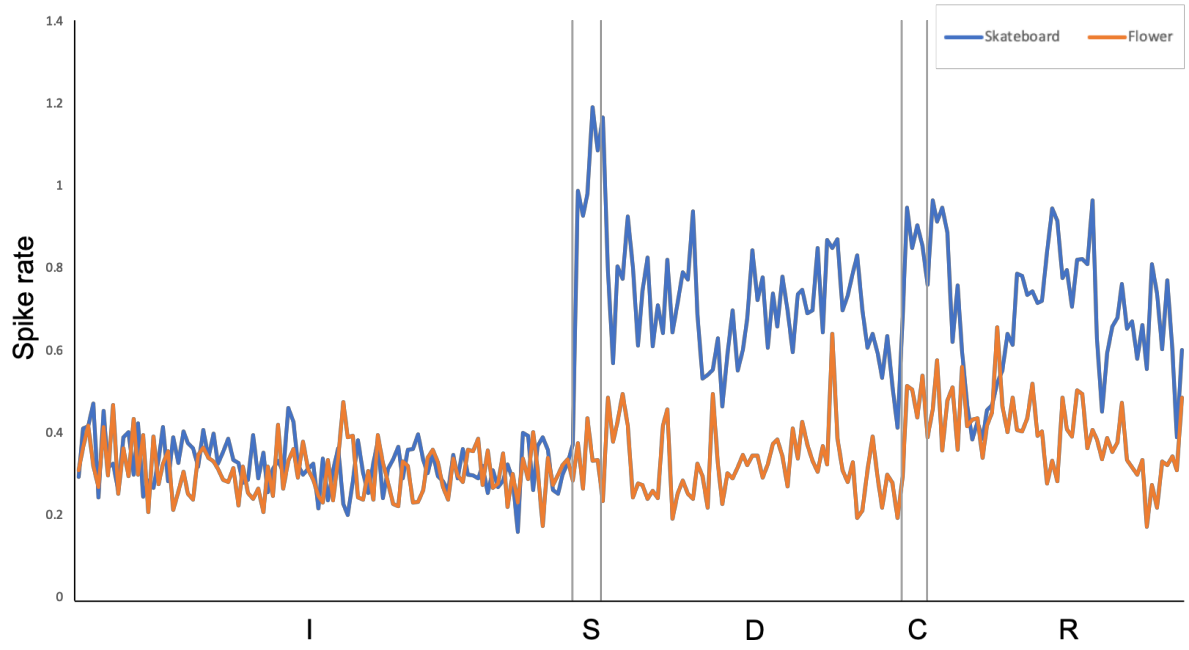

Figure 3.2: Population profile of excitatory Wulst neurons in DO. I: ITI; S: Sample; D: Delay; C: Comparison; R: Reward.

3.3.2 Excitatory CO Activity

The response profile for the excitatory delay neurons (ee, ei, en) of the CO condition is shown in Figure 3.3. In the ITI period no significant effects of stimulus $F(1,13)=0.23$, $p=0.64$, bin $F(98,1274)=1.43$, $p=0.17$, or stimulus x bin interaction $F(98,1274)=1.03$, $p=0.42$ were found. In sample period, there were no significant effects of stimulus $F(1,13)=0.99$, $p=0.34$, bin $F(5,65)=0.77$, $p=0.51$, or stimulus x bin interaction $F(5,65)=0.73$, $p=0.56$.

In the delay period, there was no significant effects of stimulus $F(1,13)=0.23$, $p=0.64$, bin $F(59,767)=1.63$, $p=0.16$, or stimulus x bin interaction $F(59,767)=1.18$, $p=0.33$.

For the comparison period, there was no significant effect of stimulus, $F(1,13)=1.54$, $p=.24$, bin $F(5,65)=0.33$, $p=0.80$, or stimulus x bin interaction $F(5,65)=1.78$, $p=0.16$ occurred. Finally, in the reward period, no significant effects of stimulus $F(1,13)=0.06$, $p=0.81$, bin $F(49,637)=2.38$, $p=0.08$, or stimulus x bin interaction $F(49,637)=1.06$, $p=0.39$ were found.

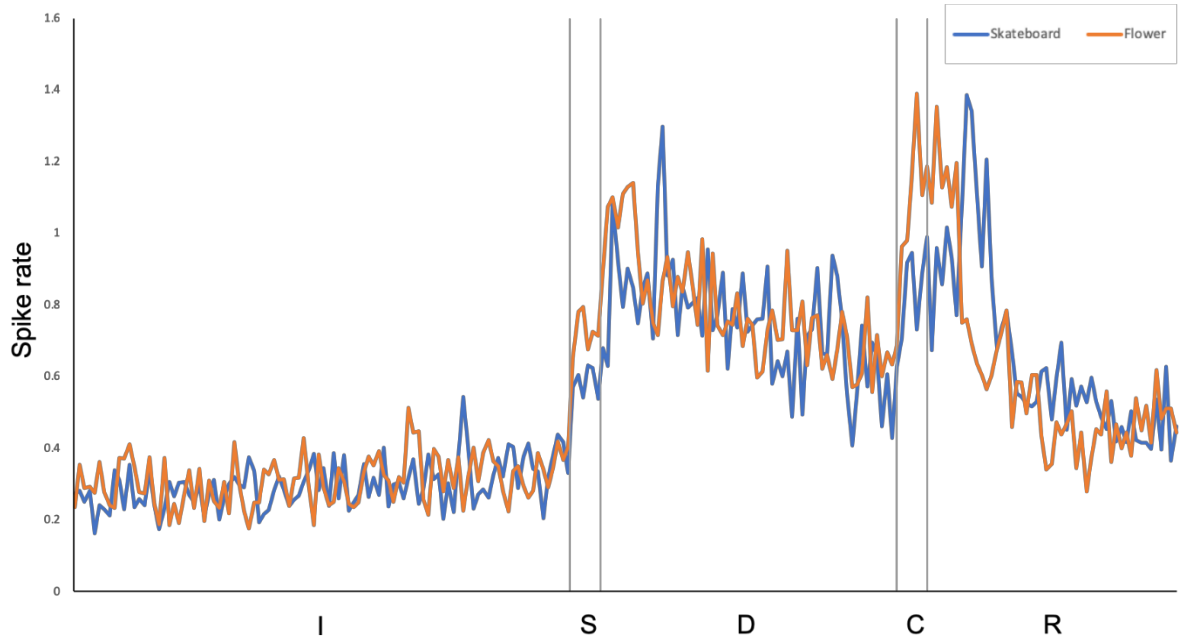

Figure 3.3: Population profile of excitatory Wulst neurons in CO. I: ITI; S: Sample; D: Delay; C: Comparison; R: Reward.

3.3.3 Inhibitory DO Activity

The response profile for the inhibitory delay neurons (ii, ie, in) of the DO condition is shown in Figure 3.4. In the ITI period no significant effects of stimulus $F(1,14)=0.84$, $p=0.38$, bin $F(98,1372)=1.53$, $p=0.14$, or stimulus x bin interaction $F(98,1372)=0.87$, $p=0.57$ were found. In the sample period, similarly there was no significant effects of stimulus $F(1,14)=0.54$, $p=0.47$, bin $F(5,70)=1.04$, $p=0.38$, or stimulus x bin interaction $F(5,70)=1.33$, $p=0.27$.

In the delay period, stimulus produced a significant effect, $F(1,14)=7.90$, $p<.014$. There was no significant effects of bin $F(59,826)=1.25$, $p=0.28$, or stimulus x bin interaction $F(59,826)=0.98$, $p=0.46$. Likewise, to the excitatory DO, the significant effect of stimulus in the delay indicates a difference between the delay activity on skateboard and flower trials. This was further explored through a comparison with the ITI activity, for which a significant difference was observed for both skateboard, $t(14)=4.12$, $p<.001$ and flower, $t(14)=8.11$, $p<.001$, again this difference was expected due to the potential reward associated with skateboard.

In the comparison period, there was no significant effects of stimulus $F(1,14)=3.02$, $p=0.10$, bin $F(5,70)=0.33$, $p=0.79$, or stimulus x bin interaction $F(5,70)=0.33$, $p=0.79$. Finally, in the reward period, no significant effects of stimulus $F(1,14)=0.23$, $p=.64$, bin $F(49,686)=1.72$, $p=0.12$, or stimulus x bin interaction $F(49,686)=1.56$, $p=0.17$. The lack of significant effect for stimulus is strange, due to pigeons' neural activity should have been being governed by the upcoming reward, similar to the excitatory DO results prior.

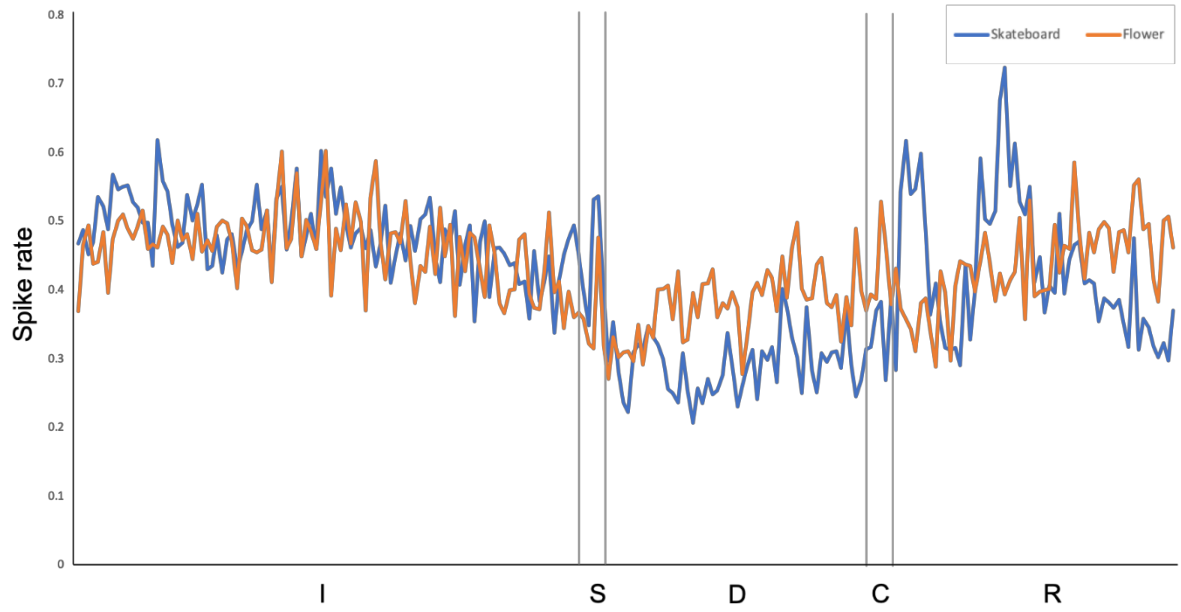

Figure 3.4: Population profile of inhibitory Wulst neurons in DO. I: ITI; S: Sample; D: Delay; C: Comparison; R: Reward.

3.3.4 Inhibitory CO Activity

The response profile for the inhibitory delay neurons (ii, ie, in) of the CO condition is shown in Figure 3.5. In the ITI period no significant effects of stimulus $F(1,8)=1.02$, $p=0.76$, bin $F(98,784)=1.18$, $p=0.33$, or stimulus x bin interaction $F(98,784)=1.13$, $p=0.36$ were found. In the sample period, there were also no significant effects of stimulus $F(1,8)=1.06$, $p=0.33$, bin $F(5,40)=1.60$, $p=0.23$, or stimulus x bin interaction $F(5,40)=0.24$, $p=0.87$.

In the delay period there was no significant effects of stimulus $F(1,8)=1.09$, $p=0.75$, bin $F(59,472)=0.43$, $p=.022$, or stimulus x bin interaction $F(59,472)=0.98$, $p=0.46$.

In the comparison period, there was no significant effects of stimulus $F(1,8)=0.16$, $p=0.70$, bin $F(5,40)=0.95$, $p=0.43$, or stimulus x bin interaction $F(5,40)=1.34$, $p=0.29$. Finally, in the reward period no significant effects of stimulus $F(1,8)=0.72$, $p=0.42$, bin $F(49,392)=0.67$, $p=0.60$, or stimulus x bin interaction $F(49,392)=1.29$, $p=0.29$ were found.

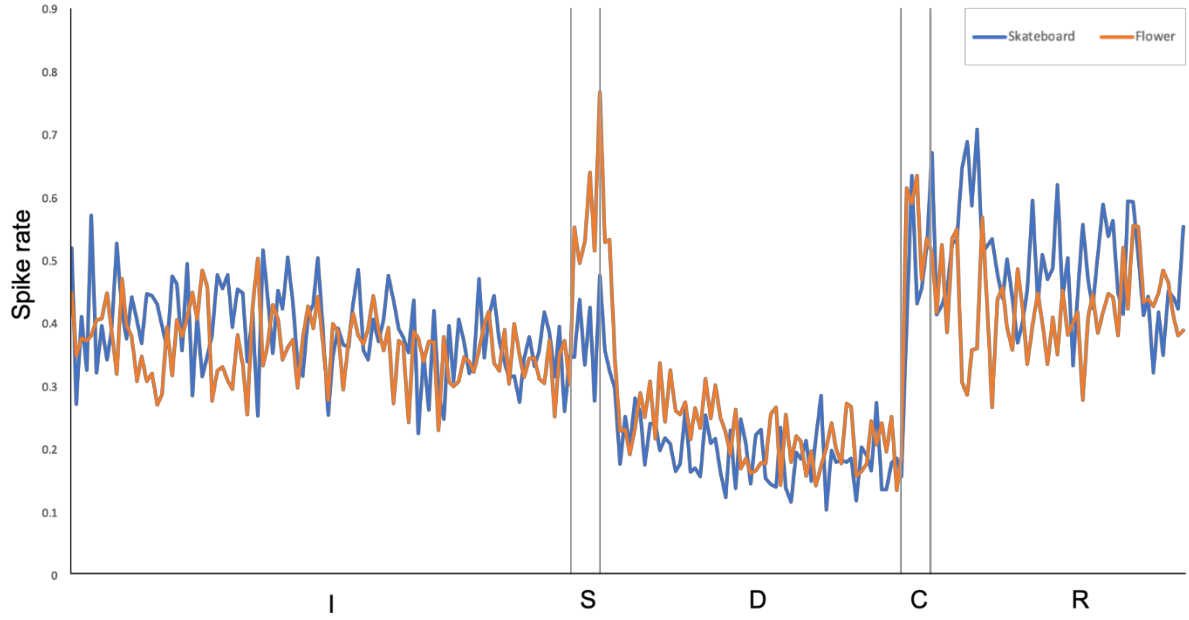

Figure 3.5: Population profile of inhibitory Wulst neurons in CO.
I: ITI; S: Sample; D: Delay; C: Comparison; R: Reward.

Chapter 4

Discussion

4.1 Summary of Results

The aim of the current study was to further our understanding of the function of the avian Wulst, an area of the brain previously linked with memory and vision (Karten et al, 1973; Pasternak, 1977). To do so, we recorded single-unit activity from the Wulst of four pigeons during a DMS task. We collected a total of 63 neurons under the DO procedure and 62 neurons under the CO procedure. A neuron was categorised as a delay neuron if there was a significant difference in neural activity between the ITI and the delay periods of skateboard trials. From the total number of neurons recorded, we found 24 delay neurons from the DO procedure and 32 delay neurons from the CO procedure.

Our hypotheses were as follows. First if the Wulst serves memory then we would expect that during the CO DMS task, delay activity would deviate from ITI activity following both skateboard and flower sample stimuli. Second, if the Wulst serves reward functions then we would expect that during the DO DMS task, delay activity would only deviate following the rewarded skateboard stimulus, but not the unrewarded flower stimulus. The response profiles of CO delay neurons were consistent with our first hypothesis that delay activity will deviate from ITI activity following both skateboard and flower sample stimuli. The response profile of inhibitory delay neurons demonstrated sustained inhibitory delay activity during both the skateboard and flower trials. Similarly, the response profile of excitatory delay neurons also demonstrated sustained excitatory activity to both skateboard and flower

stimuli. Taken together, the response profiles for the CO neurons are consistent with the idea of the Wulst being important for memory.

The response profiles of DO delay neurons were partially consistent with our hypothesis that delay activity will deviate from ITI activity following only the skateboard sample stimulus, but not following the flower sample stimuli. The response profile of excitatory delay neurons was consistent with our hypothesis, as we found sustained excitatory activity during skateboard trials but not the flower trials. The response profile of inhibitory delay neurons, however, demonstrated sustained inhibitory delay activity during both the skateboard and flower trials. This was unexpected as we predicted that only skateboard trials would exhibit activity due to only the skateboard trials being rewarded and correct flower trials not being rewarded for DO. However, skateboard trials did show a significantly greater level of inhibitory activity compared to the flower trials during the delay. Overall, delay activity during the DO procedure showed that delay activity differed between the rewarded skateboard stimulus and the unrewarded flower stimulus, which is consistent with the idea of the Wulst being important for reward processing.

4.2 Comparison to Previous Studies

Comparisons between previous studies and our study are limited due to the lack of studies that examine the neuronal properties of the Wulst. Pasternak (1977) lesioned the Wulst and found a deficit on the DMS task. This deficit was due to a decrease in the pigeons' memory and not due to perceptual disruption following surgery, as the pigeons were still able to form a preference towards one stimulus over another. Pasternak (1977) concluded that the Wulst is therefore important in working memory. Our neuronal evidence extends Pasternak's lesion findings, by also suggesting that the Wulst is involved in working memory.

Bingman, Gasser, and Colombo (2008) recorded neurons from the Wulst in pigeons trained on a visual discrimination task. Although the discrimination task is different to the DMS task, Bingman et al. (2008) found that 34% neurons were classified as delay neurons during the task. Similarly, we found that 45% of our Wulst neurons were classified as delay neurons. Unlike our study, Bingman et al. (2008) found no significant change in fire rate during the delay relative to the ITI. However, it should be noted that this delay activity during the discrimination task was present, as it was significantly greater than the activity shown during the initiation of each trial. The difference between the delay activity of the two studies may lie within the tasks used, especially as the pigeon is not expected to hold any information on the discrimination task, other than it must respond to the trial across the delay period in Bingman et al. (2008).

Comparisons across species are often difficult due to anatomical differences, however a lot of evidence suggests there are similarities between the primate IT cortex and the avian Wulst. Fuster and Jervey (1981) utilised the DMS task to show that primate higher order visual neurons could demonstrate delay activity. We were able to replicate their finding in our own study despite our use of pigeons. Although, the delay period was much longer (6 to 32 seconds) in Fuster and Jervey's study, both the IT and Wulst neurons from the two experiments showed a similar sustained activity across the delay. Furthermore, Miyashita and Chang (1988) demonstrated similar response profiles in the IT cortex during a delay non-matching to sample (DNMS) tasks. In a DNMS task, the subject is still required to hold information across a delay, therefore still requires working memory as in a typical DMS task.

4.3 Sample Or Reward Coding?

One question that arises when examining delay activity is whether the pigeons are remembering the sample stimuli or the possibility of an upcoming reward. Differentiating between sample or reward coding is not easy.

However, we are able to address the question through the use of both DO and CO procedures in the same experiment.

Johnston, Anderson and Colombo (2016), explored neural activity in the Nidopallium Caudolaterale (NCL), an area likened to the primate Pre-Frontal cortex (Rose & Colombo, 2005) and Entopallium (ENTO) a higher order visual area, in four pigeons. The study utilised the same DO procedure and DMS task as the current study. Johnston et al. (2016) found that in the ENTO, delay activity occurred following both rewarded skateboard and unrewarded flower, whereas, NCL delay activity only occurred following the rewarded skateboard and not the unrewarded flower trials. Thereby, ENTO neurons show a pattern of activity for coding the sample stimulus, consistent with primate visual cortex findings (Fuster & Jervey, 1981; Fuster & Jervey 1982, Miyashita & Chang, 1988). Conversely, the NCL neurons showed a pattern of activity associated with coding of reward, not unlike that of the primate prefrontal cortex (Rose & Colombo, 2005).

The DO excitatory delay activity from the current study showed activity similar to the NCL activity from Johnston et al. (2016), whereby the Wulst may be coding the possibility of upcoming reward as only the rewarded skateboard stimulus produced a significant change of activity across the delay. Such a conclusion is reasonable as the Wulst shares numerous reciprocal projections between the NCL, a regions highly implicated in reward processing (Shanahan, Bingman, Shimizu, Wild, & Güntürkün, 2013). Conversely, the DO inhibitory delay activity from the current study showed activity similar to the ENTO activity from Johnston et al. (2016) and Colombo et al. (2001). Due to both the rewarded skateboard and non-rewarded flower producing a significant change of activity from ITI. Such activity points to sample coding in the Wulst. Again, such a conclusion is reasonable as the Wulst is a visual area receiving input from the thalamofugal pathway (Shimizu & Bowers, 1999).

It is plausible to suggest that excitatory and inhibitory neurons might be coding different information. In this case, excitatory activity codes for reward,

whereas inhibitory activity codes for sample information. This may indeed be the case in the CO reward paradigm as well, but unfortunately we are unable to untangle the activity coding for reward and stimulus as both the skateboard and flower stimuli are rewarded. Funahashi, Bruce, and Goldman-Rakic (1989), first proposed the idea that excitatory and inhibitory activity could serve separate functions in primate vision, such that inhibitory activity may impact excitatory activity by allowing for sharper tuning of vision. In the current study it is similarly possible that the inhibitory activity is performing a similar role, by allowing for sharper tuning of the excitatory activity to code reward, as it is more salient than the visual aspect of the task.

4.4 Conclusion

Single-unit activity was recorded from the Wulst of four birds trained on a DMS task with either a DO or CO procedure. From the DO birds, we found inhibitory delay activity deviated from baseline activity following both the rewarded and the unrewarded sample stimuli, however excitatory delay activity only differed from baseline following the rewarded sample stimulus, and not the unrewarded stimulus whereas, from the CO birds excitatory and inhibitory delay activity differed from baseline activity following both of the sample stimuli. Taken together, we believe that the avian Wulst is involved in both working memory similar to the ENTO, and reward coding similar to the NCL.

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